AD_____

ARMY PROJECT ORDER NO: 87PP7863

TITLE: RETROVIRUS STUDIES IN NONHUMAN PRIMATES AT FOUR REGIONAL PRIMATE RESEARCH CENTERS

PRINCIPAL INVESTIGATOR: Leo A. Whitehair, Ph.D.

CONTRACTING ORGANIZATION: National Institutes of Health
Division of Research Resources
Bethesda, MD 20892

REPORT DATE: September 30, 1990

TYPE OF REPORT: Final Report

PREPARED FOR: U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 30, 1990	Final Report (8/20/87 - 9/30/90)

4. TITLE AND SUBTITLE

Retrovirus Studies in Nonhuman Primates at Four Regional Primate Research Centers

5. FUNDING NUMBERS

Army Project Order No. 87PP7863

63105A
3M263105DH29.AD.069
WUDA313354

6. AUTHOR(S)

Leo A. Whitehair, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

National Institutes of Health
Division of Research Resources
Bethesda, MD 20892

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick
Frederick, Maryland 21702-5012

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Progress to Date: To establish a model for the sexual transmission of AIDS, it was necessary to first show that simian immunodeficiency virus (SIV) could be transmitted across the genital mucosa of males and females.

14. SUBJECT TERMS

RAI; AIDS; SIV; Animal Models; Virology; Pathology; Lab Animals; Monkeys; Project Order

15. NUMBER OF PAGES

29

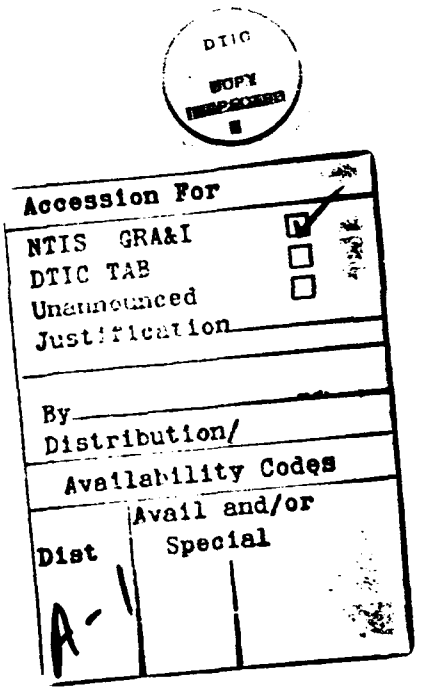
16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.



FINAL REPORT

1. Army Project No: 87PP7863

2. Report Date: September 30, 1990

3. Reporting Period: August 20, 1987 - September 30, 1990

4. NIH Contact: Dr. Leo A. Whitehair

5. Telephone No.: (301) 496-5175

6. Agency: National Center for Research Resources
National Institutes of Health

7. Project Title: Retrovirus Studies in Nonhuman Primates at Four Regional Primate Research Centers

8. Current Staff:

For the California Regional Primate Research Center (CRPRC):

Preston Marx	10%
Nicholas Lerche	15%
Jim Carlson	5%
Suganto Sutjipto	25%
Asst. Veterinarian	100%
Agengnehu Gettie	100%
Linda Antipa	100%
Sher Woods	50%
Murray Gardner, Consultant	

For the Delta Regional Primate Research Center (DRPRC):

Gary B. Baskin	50%
Louis Martin	13%
Michael Murphey-Corb	20%
Billie Fairburn	30%
Margaret R. Clarke	30%
Richard M. Harrison	10%
Dorothy Kuebler	100%
Gail Plauche	100%
Edward Moran	100%
Katherine Phillipi	100%
George Garrett	100%
Jeff Puissegur	100%
Maurice Duplantis	100%
Calvin Lanclos	10%
Donna Hollis	100%

For the New England Regional Primate Research Center (NERPRC):

Ronald D. Hunt, D.V.M.		25%	
Ronald Desrosiers, Ph.D.			10%
Norval W. King, D.V.M.		25%	
Norman L. Letvin, M.D.		10%	
Yen Li, Ph.D.		100%	
Douglas J. Ringler, D.V.M.		25%	
M.D. Daniel, Ph.D.		25%	
Keith Reimann, D.V.M.		100%	
Michael Wyand, D.V.M.		100%	
Hiroshi Tsubota, M.D.		100%	
Mark Evans, Ph.D., D.V.M.		100%	
Toshiaki Kodama, D.V.M.		100%	
Frank Tung, Ph.D.	100%		
Carol Lord	100%		
Peter DeLong		50%	
Diane Schmidt		50%	
Gregory Synder		100%	

For the Yerkes Regional Primate Research Center (YRPRC):

H. McClure	20%		
P. Fultz		20%	(6 months)
D. Anderson	20%		
A. Ansari	20%		
T. Gordon	10%		
E. White		25%	
A. Brodie	50%		
S. Klumpp	100%		
K. Paul		100%	
S. Mohammed	100%		
P. Emau		100%	(6 months)
G. Choi		100%	(6 months)
M. Saucier	100%		
M. Johnson	100%		

9. Comments for Administrative and Logistical Matters

For the CRPRC:

See letter of March 8, 1991, from Dr. Andrew G. Hendrickx to Dr. Don C. Gibson, page 2a).

For the DRPRC:

None

For the NERPRC:

None

For the YRPRC:

None

10. Scientific Progress

For the CRPRC:

SPECIFIC AIM 1.1: Prepare individual experimental immunogens for SIV core and envelope antigens and test each in rhesus monkeys for ability to produce "protective antibody" against cell-associated virus challenge. In collaboration with Dr. Paul Luciw, recombinant envelope glycoprotein, gp 120 of SIV has been expressed and produced in Chinese hamster ovary cells. The glycoprotein has been purified and immunization of rhesus macaques with purified CHO expressed gp 120 is presently in progress.

SPECIFIC AIM 1.2: To determine if actively and passively acquired SIV/SM specific antibody protects against a cell-associated challenge. These antibodies produced by psoralen-UV inactivated virus immunization, although they had neutralizing capacity failed to protect animals in an passive immunization experiment. In fact, there is suggestive evidence that antibodies accelerated the clinical disease. These experiments thus suggest that enhancement may be in vivo as well as an in vitro phenomenon. There were 4 control animals with virus alone and 4 with passive inunoglobin.

SPECIFIC AIM 1.3: To test for SIV transmission and seroconversion following inoculation of the vaginal mucosa with SIV-infected splenocytes. The Heterosexual Transmission of AIDS: A Simian Model. The overall objective of this project is to establish an animal model for the heterosexual transmission of AIDS. The model system is simian immunodeficiency virus (SIV) infection of the rhesus macaque. SIV, a lentivirus is closely related to HIV and produces an immunodeficiency syndrome in Asian macaques that is very similar to human AIDS. Two strains of SIV have been used in this study, the SIV isolate from rhesus macaques which is designated SIVMAC and an SIV isolate from the sooty mangabey monkey which is called SIVSM.

Progress to Date: To establish a model for the sexual transmission of AIDS, it was necessary to first show that simian immunodeficiency virus (SIV) could be transmitted across the genital mucosa of males and females.

Genital transmission of SIV to female rhesus macaques. We have previously reported that SIV can be transmitted across the vaginal mucosa of female rhesus macaques (Miller, et. al., 1990). To determine the effect of virus dose on the genital transmission of SIV in female rhesus macaques, cell-free SIVmac (Miller, et., al., 1990) was infused through a 2.5mm outer diameter (8 French) soft, plastic, pediatric nasogastric feeding tube (American Pharmaseal, Valencia, Ca.) into the

vaginal vault of female rhesus macaques immobilized with Ketamine HCl. After each inoculation, the animals remained immobile for 15 minutes.

To determine the infectious dose of cell-free virus required for the genital transmission of SIV and to determine if cell-free virus, in the absence of co-factors, was capable of producing infection; 4 mature female rhesus macaques were inoculated with a cell-free stock of SIVmac. Two animals received 1 ml of undiluted SIVmac virus stock (50 TCID50) and 2 animals received 1 ml of a 10-fold dilution. The inoculum was infused into the vaginal vault of the animals 8 times (twice a week for 4 weeks). SIV was isolated from the PBMC of the 2 females inoculated with the undiluted cell-free SIV by day 14 (after 4 inoculations). Of the 2 females that received the 10-fold dilution of virus, one animal was positive on day 14 (after 4 inoculations) and the other is virus negative at 20 months post-inoculation (PI). Western blot analysis of serum from these 4 animals revealed that the 3 females that became viremic were seropositive for SIV specific antibodies by 6 weeks PI while the one uninfected female has not seroconverted by 14 months PI. Of the 3 infected females, 2 are clinically normal and viremic at twenty months PI; the third was euthanized due to severe anemia and thrombocytopenia at 6 months PI.

In addition to the above group of adults, 4 juvenile female rhesus macaques were vaginally inoculated with undiluted cell-free SIVmac (50 TCID50) twice a week for 2 weeks. All 4 of the animals were virus positive by 14 days PI and all were seropositive by 4 weeks PI. At 12 months PI, two of these animals are healthy, and two animals have been euthanatized due to their poor clinical condition. At necropsy, one animal had interstitial pneumonia and enterocolitis, while the other had hepatitis, peritonitis and pancreatitis.

To determine the minimum dose of SIV needed to infect female rhesus macaques by the vaginal route, 4 females were inoculated with 1 ml of cell-free SIV (5 TCID50) once on day 13 of the menstrual cycle. All 4 females remained virus negative and seronegative for 6 months. After 4 months, the same animals were again inoculated with 1 ml of cell-free SIV (50 TCID50) once on day 13 of the menstrual cycle. Three of the animals became viremic by 14 days PI and these animals were seropositive by 4 weeks PI. One animal is virus negative and seronegative at 4 months PI. All the animals are clinically normal at 12 months PI. In ~~addition, 9 more mature female rhesus have been inoculated with 1 ml of~~ cell-free SIVmac (50 TCID50) and 7 were viremic by 14 days PI. Two animals are virus negative and seronegative at 9 months PI. Thus, 10 of 13 female rhesus vaginally inoculated once with cell-free SIV mac became infected.

Effect of nonoxynol-9 containing spermicide on the genital transmission of SIV in female rhesus macaques. In an attempt to prevent the genital transmission of SIV to female rhesus macaques, 1 ml of contraceptive foam containing nonoxynol-9 (12.5% v/v) was infused into the vaginal canal of 6 animals just prior to the inoculation of 1 ml of cell-free SIV (50 TCID50). This sequence was repeated a total of 4 times over 2

weeks. (This dose of SIV had previously produced infection in 4 of 4 females.) Three of 6 animals were virus positive at 2 weeks PI and seropositive at 4 weeks PI. The other 3 animals are virus negative and seronegative at 15 months PI. Thus, nonoxynol-9 containing spermicides provide partial protection against the genital transmission of SIV.

Genital transmission of SIV to male rhesus macaques. To transmit SIV to male rhesus macaques by the genital route, cell-free SIVmac was gently infused onto the urethral mucosa of immobilized males by inserting a 2.5mm outer diameter (8 French) soft, plastic, pediatric nasogastric feeding tube (American Pharmaseal, Valencia, Ca.) approximately 1 cm into the urethra. Although the animals remained immobile for 15 minutes, some of the inoculum drained from the urethra and came into contact with skin surrounding the urethral orifice. To infect males with SIV, two mature male rhesus macaques were inoculated with 1 ml of cell-free SIVmac (25 TCID50) once a week for three weeks. Both animals became viremic by 31 days PI. One male had a strong antibody response to SIV antigens by 6 weeks PI. This animal developed lymphadenopathy and splenomegaly but was alive and viremic at 30 months post-inoculation. In contrast, the other male developed only a weak antibody response and at 6 months PI became moribund with persistent diarrhea and severe wright loss. At necropsy, this animal had disseminated cytomegalovirus infection, ulcerative gastritis and severe interstitial pneumonia with multinucleate giant cells.

In addition to the above group of adult males, 4 juvenile male rhesus macaques were inoculated intraurethrally with cell-free SIVmac (50 TCID50) twice a week for 2 weeks. All were viremic by 14 days PI and seropositive by 4 weeks PI. All four animals are healthy at 10 months PI.

To determine if it was possible to transmit SIV to males by placing the virus on the intact skin of the penis, 1 ml of cell-free SIVmac (50 TCID50) was placed on the foreskin and glans of the penis of 4 mature male rhesus macaques. Some of the virus inoculum came in contact with the mucocutaneous junction of the urethral os. This procedure was performed twice in 5 days. Two of these animals were viremic by 14 days PI and seropositive by 4 weeks PI. The 2 others are virus negative and seronegative at 9 months PI.

These results show that male and female rhesus macaques can be infected via the genital mucosa. Furthermore, the disease that eventually developed in one male and 2 females was indistinguishable from that seem in intravenously inoculated animals.

Isolation of SIV from genital secretions. Vaginal secretions from 5 SIV-infected female rhesus macaques were collected three times (once every 2 weeks for 6 weeks) and cultured. To isolate virus from vaginal secretions, the vaginal vault was lavaged with 2 mls of RPMI 1640 (containing 1 mg/ml Amphotericin B) and the wash was collected. Samples contaminated with menstrual blood were discarded. The mononuclear cells in the lavage fluid were separated by gradient centrifugation on Ficoll-

Hypaque, washed and cultured for SIV as described previously (Miller, et. al., 1990). The supernatant fluid was collected, filtered through a 0.45 micron filter and cultured for SIV as described (Miller, et. al., 1990). Vaginal secretions from 5 SIV-infected female rhesus macaques were collected three times (once every 2 weeks for 6 weeks) and cultured. Samples contaminated with menstrual blood were discarded. SIV was isolated from the cells and cell-free filtrates from the vaginal secretions of 2 of 5 females on the first collection, from the cells and filtrate from 1 of 4 animals on the next collection, and from the cells of one female and the cells and filtrates of 2 females on a third collection. The positive samples were collected at all stages of the menstrual cycle and all the animals were healthy and viremic. these findings suggest that SIV can be shed at any stage of the menstrual cycle and the fact that virus was never recovered from the vaginal secretions of 2 animals suggest that individual factors may be important in determining if SIV is present in vaginal secretions.

We have previously reported that SIV can be isolated from the semen of SIV-infected male rhesus macaques (Miller, et. al. 1990).

Significance. The initial objective of this project was to determine if a nonhuman primate model for the sexual transmission of AIDS could be developed in the SIV/rhesus macaque model of AIDS. Our findings show that rhesus monkeys can be infected via the genital tract and that this model can be further developed to test the role of chemical and pharmacologic contraceptives as well as surgical contraceptive procedures in the prevention/enhancement of HIV transmission in humans. This model will have an impact on AIDS research of others in that a practical model for studying the sexual transmission of AIDS is feasible and being developed.

We have defined the dose of cell-free SIV necessary for genital transmission in female rhesus macaques. By using the smallest challenge dose of cell-free SIV that is known to produce viremia in 100% of inoculated animals, we can more precisely assess the ability of spermicides and other agents to prevent the genital transmission of SIV.

We have begun to use the animal model we developed to explore specific strategies to prevent the heterosexual transmission of HIV. We have shown that spermicides containing nonoxynol-9 do provide partial protection against the genital transmission of SIV to female rhesus macaques. Although it has been previously shown that N-9 can inactivate HIV in vitro, this is the first attempt to stop the genital transmission of SIV using N-9.

The finding that SIV can be transmitted to male macaques by placing the virus on the skin of the penis, suggests that it may be easier to transmit HIV to males by heterosexual contact than has been appreciated.

SPECIFIC AIM 1.4: Assessment of the ability of SIV to infect New World monkey species.

This study was completed and results were given in a separate report.

SPECIFIC AIM 1.5: To put in place a simian retrovirus reference laboratory to screen human and non-human primate sera for antibodies against SIV, STLV, and SRV, and to seek new isolates for captive and wild populations of non-human primates. This laboratory is now well established, having tested over 10,000 non-human primate sera for retorviral antibodies. Approximate overall prevalences for the three virus groups are as follows. SIV: 0% in macaques, 8-10% in African species. STLV: 10% in macaques, 17% in African species. SRV: 5% in macaques, 0% in African species. The major focus of the laboratory is now to provide serologic testing and virus isolation in support of NIH contract facilities for the development of specific pathogen-free (SPF) rhesus monkey breeding colonies. Activities of the laboratory have led to new SIV isolates from a sooty mangabey in West Africa, and from a captive mandrill.

Publications

Miller C.J., Alexander N.J., Sutjipto S., Lackner A.A., Hendrickx A.G., Gettie A., Lowenstine L.J., Jennings M., and Marx P.A. Genital mucosal transmission of simian immunodeficiency virus: Animal model for heterosexual transmission of human immunodeficiency virus. J. Virology, 63:4277-4284, 1989. (Appendix A)

Marx P.A., Miller C.J., Alexander N.J., Sutjipto, S., Lackner A.A., Gettie A., Jennings M., and Hendrickx A.G. An animal model for sexual transmission of HIV. In Heterosexual Transmission of AIDS. Eds. N. Alexander, H.L. Gablenick, and J.M. Spieler. Alan R. Liss, Inc., 1990.

In Press

Miller C.J., Alexander N.J., Sutjipto S., Joye S.M., Hendrickx A.G., Jennings M., and Marx P.A. Effect of virus dose and nonoxynol-9 on the genital transmission of SIV. J. of Medical Primatology. In press.

For the DRPRC:

SPECIFIC AIM 2.1: Serial Sacrifice Studies

We are continuing to study the tissues from all of the sacrificed animals by immunohistochemistry for viral and cell-surface antigens, by electron microscopy, and by *in situ* hybridization. During the period of this report we completed studies on the thymus, lung, brain, and liver and submitted these results for publication.

The thymuses from 20 SIV-infected and 4 uninfected rhesus monkeys were examined at intervals after infection to determine whether there were specific SIV-induced lesions, to document the serial distribution of SIV

antigens, mRNA, and DNA, to quantitate the number of infected cells, and to correlate thymic changes with other parameters of infection. The following techniques were used: gross pathology, histopathology, immunohistochemistry, electron microscopy, in situ hybridization, polymerase chain reaction, and limiting dilution culture. Thymic involution due to loss of lymphocytes was apparent 8 weeks after inoculation. No epithelial damage or loss of Hassall's corpuscles was observed. Culture was the most sensitive technique for detecting SIV, being positive in 19 of 20 inoculated monkeys. The polymerase chain reaction was negative in one thymus that was positive at a low level by culture. In situ hybridization was positive in 14 of 19 thymuses examined, with a few macrophages in the cortex having a strong signal and numerous lymphocytes in the medulla having a weak signal. Mature viral particles and viral budding could not be demonstrated by electron microscopy. The number of cells positive for viral RNA by in situ hybridization correlated with the level of serum antigenemia.

These observations suggest that thymic macrophages and lymphocytes are infected with SIV within two weeks after inoculation. SIV apparently directly causes loss of thymic lymphocytes and immunodeficiency without infecting or damaging the thymic epithelium. No specific SIV-induced lesions were recognized. The number of cells in the thymic medulla expressing SIV RNA correlates with the level of serum antigen, which has been previously shown to be correlated with disease progression.

The lungs from 24 rhesus monkeys that had been experimentally infected with simian immunodeficiency virus, that were free from cytomegalovirus and rhesus Epstein-Barr virus, and that had no etiologic agent other than SIV detected in the lung were studied. The following lesions were detected: perivascular inflammation, vasculitis, interstitial pneumonia, syncytial cells, hemorrhage, fibrin exudation, and pleural fibrosis. The lesions appeared to be dirctly due to SIV infection. SIV antigens, RNA, and virions were detected in syncytial cells and macrophages by immunohistochemistry, in situ hybridization, and transmission electron microscopy respectively.

The serial study of early SIV brain infection revealed initial involvement of leptomeninges, followed by perivascular infection of brain parenchyma. Severity of SIV encephalitis correlated with severity of systemic disease rather than with length of infection. Diffuse white matter disease was not observed, and there was little evidence of SIV infection of brain endothelial cells. SIV infection of leptomeninges is separate from infection of the brain, which appears to be due to transvascular spread of infected monocytes/macrophages.

Publications Supported in Part by Army Funds:

Hu, F-S, Baskin, GB, Murphey-Corb, M, and Kuebler, D. Localization of simian immunodeficiency virus in serially sacrificed rhesus monkey tissues by *in situ* hybridization. J Cell Biochem (Suppl 14, pt D): 66, 1990 (Abstract)

Sharer, LR, Michaels, J, Murphey-Corb, M, Hu, F-S, Kuebler, DJ, and Baskin, GB. Serial pathogenesis study of SIV brain infection. Eighth Annual Symposium Non-Human Prim Models AIDS, Abstract #20, 1990 (Abstract)

Sharer, LR, Michaels, J, Murphey-Corb, M, Hu, F-S, Kuebler, DJ, Martin, LN, and Baskin, GB. Serial pathogenesis study of SIV brain infection. J Med Primatol (In press)

Baskin, GB, Murphey-Corb, M, Martin, LN, Soike, KF, Hu, F-S, and Jeubler, D. Lentivirus-induced pulmonary lesions in rhesus monkeys infected with SIV. (Submitted)

Baskin, GB, Murphey-Corb, M, Martin, LN, Davison-Fairburn, B, Hu, F-S, and Kuebler, D. The thymus in SIV-infected rhesus monkeys. (Submitted)

Gerber, MA, Chen, M-L, Hu, F-S, and Baskin, GB. Liver lesions in rhesus monkeys infected with simian immunodeficiency virus. Am J Pathol. (Submitted)

SPECIFIC AIM 2.2: Maternal-Fetal Transmission Studies

Female rhesus monkeys were inoculated with SIV 1-2 months before breeding to allow pregnancy during a fully immunosuppressed state and thereby enhance transplacental transfer of SIV. It was planned that half of the pregnant females would be delivered by caesarian and the other half normally to further evaluate the role of parturition during vaginal delivery on perinatal infection. Sixteen female rhesus monkeys purchased from the FDA were inoculated with 10 i.d.50 SIVDeltaB670 during 10/15/90-11/6/90. Parings with males after progesterone withdrawal was attempted monthly for 4 months. Only one successful pregnancy was accomplished by this procedure, a factor likely due to the age and clinical condition of the females.

One healthy latently infected female was also successfully impregnated. She will be allowed to deliver vaginally and the baby will remain with the mother after birth. This animal is being followed weekly for an increase in viral activity by PCR, coculture, and assessment of antigenemia to determine the role, if any, of hormones induced by pregnancy and lactation on interruption of virus latency.

Five uninfected females were paired monthly for 4 months with infected, viremic males to assess the incidence of male to female transmission of SIV. Although 4 of 5 females were successfully impregnated, none became infected as a result of the matings.

The 4 females from the male to female transmission study, and 4 females matched for gestational age by ultrasonography taken from the breeding colony, have been inoculated during early third trimester with either SIV/DeltaB670, a highly pathogenic isolate, or BK-28, an infectious molecular clone of SIVmac that is minimally pathogenic. All females will be allowed to deliver vaginally and infants will be removed from

the mother immediately after birth. This study will address the relevance of viral virulence on the production of viable infected offspring since we have shown previously that 1 of 4 females infected with B670 during pregnancy aborted. The vaginal delivery will determine if the rate of neonatal infection increased with normal delivery relative to caesarian delivery.

We have also assigned 8 healthy, young females with an active breeding history for the next fall breeding cycle to repeat the experiment described above.

SPECIFIC AIM 2.3: Passive Protection with Monocional Antibodies Studies

None

SPECIFIC AIM 2.4: Rhesus Breeding for SAIDS Research

There were 325 animals in the SAIDS breeding program at the beginning of the reporting period, and 376 animals at the end of the reporting period. Sixteen animals were transferred to research projects during this time period.

The Chinese origin rhesus population consisted of 168 animals on January 1, 1990, and 204 animals on August 31, 1990. There were a total of 63 pregnancies out of 94 adult females (67%). There were 6 stillbirths and abortions, 3 spontaneous deaths, and 3 neonatal deaths, for a total of 52 surviving offspring (81% survivorship). Ten adult females died during this time period. Infants were made available for SAIDS research, but none were transferred during this time.

The Indian origin rhesus monkey breeding colony consisted of 157 animals on January 1, 1990, and 172 animals on August 31, 1990. There were 51 pregnancies out of 82 adult females (62%). There were 2 stillbirths, one spontaneous death, and two neonatal deaths, for a total of 46 surviving offspring (90% survivorship). Nine adult females and two adult males died during this reporting period. Sixteen infants were transferred to the SAIDS nursery for hand-rearing. Since a limited number of Indian rhesus breeding females are available in the United States, we will retain these female infants as future breeders. The males are available for SAIDS research use.

The mortality patterns in the two colonies were much more similar than reported in previous reports, suggesting that the increased mortality in the aging Indian rhesus colony has balanced out. There were only 2 trauma deaths in each colony, all associated with social reorganizations. Funds for expanding the Indian rhesus colony have been awarded, and we expect production to increase in 1992.

Routing care and inventory have been completed. Animals housed in corncribs are being trained to run into chutes to minimize capture stress. Animals in corrals are already trained to run into chutes on

the attached catchpen. Behavioral observations include male social integration, and dominance and affiliative interactions of females.

Publications:

Phillippi, K.M., Clarke, M.R., and Blanchard, J.L., 1990. Survey of apthogenic and non-pathogenic parasites of rhesus monkeys housed in small social groups. Amer. J. Primatol. 20:221. (Abstract)

Zucker, E.L., Mayeaux, D.J., Phillippi, K.M., and Clarke, M.R., 1990. Interactions of male Chinese rhesus monkeys while in an all-male group and in breeding groups. Amer. J. Primatol. 20:248. (Abstract)

For the NERPRC:

SPECIFIC AIM 3a.: Characterization of the Humoral and Cellular Immune Response to SIV in terms of antibody Specifications and the Various Components of Cellular Immunity

We have cloned and sequenced a rhesus monkey major histocompatibility complex (MHC) class I gene and shown that protein expressed from this gene binds a 9 amino acid fragment of the SIVmac gag protein and presents that peptide to cytotoxic T lymphocytes (CTL). We have then employed this understanding of the SIVmas-specific CTL response to show that a vaccinia-SIVmas gag viral construct can induce long-lasting gag-specific CTL in rhesus monkeys. This was the first demonstration of a vaccine-induced CTL response to an AIDS virus.

SPECIFIC AIM 3b.: Determination of the basis for SIV persistence

Six monoclonal antibodies were identified that recognize SIVmac gp120 (SU) and that neutralize SIVmac infectivity. These six neutralizing monoclonal antibodies (NMAbs) fall into at least three distinct groups on the basis of competition assays. At least five of the six NMAbs appear to recognize discontinuous or conformational epitopes. Four of them did not recognize denatured antigens in western blot and they did not react with E. coli recombinant fragments. None of the five recognized smaller peptides in pepscan analysis. In order to define the determinants of recognition by these NMAbs, the reactivities of gp120 from six different molecular clones of defined sequence were analyzed. SIVmac251, SIVmac239, and three variants of SIVmac239 (1-12, 8-22, and 3-18) are all closely related with only 17 to 28 amino acid differences per gp120. The three variants of SIVmas239 evolved during the course of persistent infection in vivo (see J. Virol. 65, 1843, 1991 and previous report). SIVmac142 is somewhat more distant. Distinct patterns of reactivity were observed.

	50.1	101.1	71.1	KK5	KK9	KK17	
SIVmas251	++	++	++	++	++	++	Reactivity of
SIVmac142	-	-	-	-	-	++	gp120 by
SIVmac239	-	+	++	++	+	++	Radioimmuno-

SIVmac239/1-12	-	-	(+)	-	-	-	precipitation
SIVmac239/8-22	-	(+)	(+)	+	-	+	(+) = very weak
SIVmac239/3-18	-	(+)	++	++	+	++	reactivity

Our results indicate that conformational or discontinuous epitopes are important determinants for recognition of antibodies that neutralize SIVmac infectivity. Furthermore, sequence changes in the variable domains of SIVmacgp120, that accumulate during persistent infection in vivo, dramatically influence recognition by neutralizing antibodies. Finer recombinants and site specific mutants are being used to define the variable domains important for recognition by these NMAbs.

SPECIFIC AIM 3c. Determination of the pathogenesis of SIV-induced disease with emphasis on interactions of virus, origin, and cellular tropisms and host immune response

Previously, we reported that tissue macrophages from SIV-infected animals were capable of supporting SIV replication in vivo and in vitro and expressed altered immunophenotypes (Ringler, et al, Lab. Invest., 62:435, 1990; Ringler, et al, Am. J. Pathol. 134:373, 1989). We have continued these studies on the influence of SIV on the tissue macrophage. We have found that infection of alveolar macrophages with SIV in vitro does not cause them to produce elevated levels of TNF-*a*, an important immunoregulatory cytokine, nor does infection cause them to be hyperresponsive to endotoxin, in terms of TNF-*a* production. However, alveolar macrophages retrived from asymptomatic animals infected with SIV produce elevated levels of TNF-*a* upon endotoxin challenge as compared to alveolar macrophages retrieved from normal animals. These results suggest that animals or people infected with AIDS viruses may be predisposed to systemic effects of elevated levels of TNF-*a*, with inflammatory sequelae. This work is in press in Lab. Invest. (Horvath, et al).

In addition, we have studies the effects of certain cytokines on SIV replication in alveolar macrophages in vitro. As reported for HIV-1 replication in macrophages, TNF-*a* significantly increased SIV gag protein in culture supernatants. However, after correcting for differences in total cell numbers and numbers of gag-containing cells in the trested and untreated cultures, GMCSF did not upregulate SIV production on a per cell basis. IL-6 increased SIV replication little, if at all, but induced significantly greater cytopathic changes in the treated cultures compared with infected, untreated cultures. In contrast, IFN-*r* greatly decreased replication. Our results for GMCSF, IFN-*r*, and IL-6 are in contrast to previously published reports of cytokine control of HIV-1 growth in target cells, and they stress the importance of cell number analyses and the use of primary cultures in the study of lentiviral replication kinetics in macrophages.

SPECIFIC AIM 3d.; Genetic Basis for SIV Tropism

Macrophage tropic virus variants evolved during the course of infection of individual rhesus monkeys with cloned, non-macrophage tropic simian immunodeficiency virus (SIV). Specific changes in the envelope gene (*env*) were found to be primarily responsible for the dramatic increase in viruses ability to replicate in macrophages. Cloned viruses differing at nine amino acid positions in *env* exhibited more than a 100 fold difference in replicative capacity for primary cultures of rhesus monkey alveolar macrophages. At least five of the nine amino acid changes contributed to macrophage tropism. These determinants were distributed across the full length of *env*, including both the gp120 and gp41 products of the *env* gene. Furthermore, the emergence of macrophage tropic variants in vivo was associated with specific pathologic manifestations in which the macrophage is the major infected cell type. Thus, major determinants of macrophage tropism reside in *env*, they can be complex in nature and the presence of macrophage tropic virus variants in vivo can influence the disease course and disease manifestations.

SPECIFIC AIM 3e.: Evaluation of an approach to SIV vaccine development using a defective virus

A 3.5kb SacI fragment encompassing env gene sequences of cloned SIVmac239 DNA was subcloned into the retrovirus vector pZipneoSV(X). A 5.6 kb NarI-SphI fragment of cloned SIVmac239 DNA encompassing gag, pol, vif, vpx, and vpr was also subcloned into the same retrovirus vector. The orientations of the inserts were identified by restriction endonuclease mapping. The sense orientation constructs were designated SIVgp$^+$ (gag-pol containing) and SIVenv$^+$ (env containing). Similarly, antisense constructs were designated SIVgp$^-$ and SIVenv$^-$. All constructs were transfected into the amphotropic packaging cell line GPenvAM12 (kindly provided by Dr. Arthur Bank of Columbia University). The packaging cell line provided all the necessary viral proteins to encapsidate retroviral RNA. High titer virus stocks were harvested (10^5 - 10^6 cfu/ml). Four animals were inoculated with sense construct recombinant virus (5 X 10^5 cfu) and antibody response to the recombinant virus infection was monitored by ELISA.

		Day 0	Wk2	Wk4	Wk6	Wk8	Wk10	Wk12	Wk15
SIVgp$^+$	Mm181-88	0.023	0.054	0.106	0.051	0.117	0.077	0.066	0.025
	Mm230-88	0.010	0.023	0.044	0.038	0.095	0.077	0.152	0.056
SIVenv$^+$	Mm277-88	0.080	0.211	0.126	0.095	0.130	0.083	0.075	0.040
	Mm356-88	0.013	0.344	0.465	0.474	0.573	0.198	0.188	0.119

These results suggest that MM 356-88 may have produced anti-SIV antibodies in response to exposure to the SIVenv$^+$ retrovirus vector. These results will need to be confirmed by other specific tests.

In the last progress report, we discussed our findings that antisense constructs of *gag-pol* and *env* of SIV were able to inhibit uncloned SIVmac virus replication in the retrovirus vector transduced HuT-78 cells. Now we report our findings that the sense construct of SIV *gag-pol* transduced HuT-78 cells were protected from HIV-1 and HIV-2 infection. The mechanisms of this protection are under investigation.

SPECIFIC AIM 3f.: Development of infectivity assays for SIV by the intravenous, oral, vaginal and other routes.

Data described in a previous progress report failed to implicate the passage of SIV through intact squamous mucosa. We have documented that SIV can traverse across abraded squamous mucosal surfaces.

Publications that resulted totally or in part from this support:

Horvath, CJ, Desrosiers, RC, Sehgal, PK, King, NW, and Ringler, DJ. Effect of simian immunodeficiency virus infection on tumor necrosis factor-a production by alveolar macrophages. Lat. Invest., in press, 1991.

Ringler, DJ, Wyand, MS, Walsh, DG, MacKey, JJ, Chalifoux, LV, Popovic, M, Minassian, AA, Sehgal, PK, Daniel, MD, Desrosiers, RC, and King, NW. Cellular localization of simian immunodeficiency virus in lymphoid tissues. Am. J. Pathol., 134-373-383, 1989.

Ringler, DJ, Walsh, DG, MacKey, JJ, Chalifoux, LV, Daniel, MD,, Daniel, MD, Desrosiers, RC, King, NW, and Hancock, WW. Soluble and membrane-associated interleukin 2 receptor-a expression in rhesus monkeys infected with simian immunodeficiency virus. Lab. Invest., 62:435-443, 1990.

Walsh, DG, Horvath, CJ, Hansen-Moosa, A, MacKey, JJ, Sehgal, PK, Daniel, MD, Desrosiers, RC, and Ringler, DJ. Cytokine influence of SIV replication with primary macrophages. Am. J. Pathol., in press, 1991.

Kannagi, M, Chalifoux, LV, Lord, CT, and Letvin, NL. Suppression of simian immunodeficiency virus replication in vitro by CD8+ lymphocytes. J. Immunol. 140:2237-2242, 1988.

Tsubota H, Lord, CI, Watkins, DI, Morimoto, C, and Letvin, NL. A cytotoxic T lymphocyte inhibits AIDS virus replication in peripheral blood lymphocytes. J. Exp. Med. 169:1421-1434, 1989.

Miller, MD, Lord, CI, Stallard, V, Mazzara, GP, and Letvin, NL. Gag-specific cytotoxic T lymphocytes in rhesus monkeys infected with the simian immunodeficiency virus of macaques. J. Immunol. 144:122-128, 1990.

Yamamoto, H, Miller, MD, Tsubota, H, Watkins, DI, Mazzara, GP, Stallard, V, Panicali, DL, Aldovini, A, Young, RA, and Letvin, NL. Studies of cloned simian immunodeficiency virus-specific T lymphocytes: Gag-specific cytotoxic T lymphocytes exhibit a restricted epitope specificity. J. Immunol. 144:3385-3391, 1990.

Yamamoto, H, Miller, MD, Watkins, DI, Snyder, GB, Chase, NE, Mazzara, GP, Stallard, V, Panicali, and D, Letvin, NL. Two distinct lymphocyte populations mediate simian immunodeficiency virus envelope-specific target cell lysis. J. Immunol. 145:3740-3746. 1990.

Miller, MD, Yamamoto, H, Hughes, AL, Watkins, DI, and Letvin, NL. Definition of an epitope and MHC class I molcular recognized by gag-specific cytotoxic T lymphocytes in SIVmas-infected rhesus monkeys. J. Immunol. 147:320-329, 1991.

Shen, L, Chen, ZW, Miller, MD, Stallard, V, Mazzara, GP, Panicali, DL, and Letvin, NL. Induction of simian immunodeficiency virus-specific CD8+ cytotoxic T lymphocytes following vaccination with a recombinant virus vaccine. Science 1991; in press.

Kestler, H, Kodama, T, Ringler, D, Marthas, M, Pedersen, N, Lackner, A, Regier, D, Sehgal, P, Daniel, M, King, N, and Desrosiers, RC. Indication of AIDS in rhesus monkeys by molecularly cloned simian immunodeficiency virus. Science 248:1109-1112, 1990.

Burns DPW, and Desrosiers, RC. Sequence variability of simian immunodeficiency virus in a persistently infected rhesus monkey. J. Med. Primatol. 19:317-326, 1990.

Regier, DA, and Desrosiers, RC. The complete nucleotide sequence of a pathogenic molecular clone of simian immunodeficiency virus. AIDS Res. Hum. Retroviruses 6:1221-1231, 1990.

Burns, DPW, and Desrosiers, RC. Selection of genetic variants of simian immunodeficiency virus i persistently infected rhesus monkeys. J. Virol. 65:1843-1854, 1991.

Kodama, T, Burns, DPW, Silva, DP, diMarzo Veronese, F, and Desrosiers, RC. Strain-specific neutralizing determinant in the transmembrane protein of simian immunodeficiency virus. J. Virol. 65:2010-2018, 1991.

Desrosiers, RC, Hansen-Moosa, A, Mori, K, Bovier, DP, King, NW, Daniel, MD, and Ringler, DJ. Macrophage-tropic variants of SIV are associated with specific AIDS-related lesions but are not essential for the development of AIDS. Am. J. Pathol. 139:1-7, 1991.

Kestler, HW, III, Ringler, DJ, Mori, K, Panicali, DL, Sehgal, PK, Daniel, MD, and Desrosiers, RC. Importance of the *nef* gene for maintenance of high virus loads and for the development of AIDS. Cell 65, 651-662, 1991.

For the YRPRC:

SPECIFIC AIM 3a: Monitoring Chronically Infected Macaques

We have monitored a group of 13 macaques (12 rhesus and 1 pigtail) experimentally infected with SIVsmm for a period of 54 to 66 months. These animals developed variable degrees of lymphadenopathy, splenomegaly, diarrhea, weight loss, and hematologic abnormalities, including lymphopenia, neutropenia and thrombocytopenia. Eight of these 13 chronically infected macaques (61.5%) died from an AIDS-like disease between 14 and 43 months post-infection, and one additional animal continues to show immunosuppression, periodic episodes of diarrhea and poor wright gain. All clinically ill animals have shown progressive decreases in CD4+ cells and in their CD4+/CD8+ cell ratios. Sentinel animals housed in the same room or same cage with macaques chronically infected with SIVsmm have remained seronegative and virus negative. These observations indicate that the disease induced by SIVsmm infection in macaques is remarkably similar to human AIDS, and that SIVsmm, like HIV, is not transmitted by casual contact. These extended observations also suggest that SIVsmm infection in macaques may not always result in fatal disease as was initially believed. Animals which survive such infections should be extensively evaluated to attempt to determine the reasons for their ability to resist fatal infection.

SPECIFIC AIM 3b.: Isolation of SIV from Stumptail Macaques

As noted in our previous progress report, widespread lentivirus infection has been documented in a stumptail macaque colony at the Yerkes Center. This colony showed a high morbidity and mortality rate over a period of approximately two years, with severe weight loss and opportunistic infections (candidiasis, mycobacteriosis, and CMV infection) as major necropsy findings. These animals have shown significant reductions in lymphocyte counts, reduced number of $CD4^+$ cells and decreased $CD4^+/CD8^+$ cell ratios. This isolate has been cloned and sequenced (Drs. P. Johnson and V. Hirsch), and groups of rhesus and pig-tailed macaques have recently been inoculated with either cloned or uncloned SIVstm isolates to determine the pathogenicity and pathogenesis of infection in other macaque species.

SPECIFIC AIM 3c.: SIVsmm and SYLV-1 Infection in Sooty Mangabeys in the Yerkes Colony.

Studies are continuing in the Yerkes Center's sooty mangabey breeding colony to determine the incidence of natural infection with both SIVsmm and STLV-1, age at time of infection, and modes of transmission within the colony. A small group of virus negative mangabeys has been removed from the main mangabey colony to establish a retrovirus-free mangabey breeding colony. Overall, 62% of the mangabey colony is positive for SIVsmm infection and 43% of the colony is seropositive for STLV-1. The frequency of SIVsmm infection in the mangabey colony increases with age

of the animal; infection has been documented in 94% of mangabeys nine years of age or older, 83% of animals 7-8 years old, 73% of 5-6 year old animals, 49% of 3-4 years old, and in 23% of animals 1-2 years of age. Although natural SIVsmm and/or STLV-1 infection in the mangabey does not usually result in clinical disease, recent cases of lymphoma or leukemia have been documented in mangabeys infected with both SIVsmm and STLV-1.

SPECIFIC AIM 3d.: Perinatal Transmission of SIVsmm

Studies to evaluate the perinatal transmission of SIVsmm were initiated by infecting 15 timed pregnant rhesus monkeys with SIVsmm during various stages of gestation and monitoring the offspring for evidence of virus infection. Three groups of five animals were infected with SIVsmm during early (day 28-35), mid (day 71-78), and late (day 146-150) gestation. Offspring delivered by these experimentally infected macaques included 2 stillbirths and 13 livebirths; 1 liveborn infant died at 3 days of age. There was no evidence of virus infection in the stillbirths or neonatal deaths. The remaining infants and their mothers were evaluated within a week of parturition and at quarterly intervals thereafter by serology and virus culture of PBMC; milk samples were also collected from the mothers at each examination for virus culture. All infants were virus negative at birth; all infants in the early and mid-gestation groups and one infant in the late gestation group had low levels of maternal antibodies to SIVsmm. These maternal antibodies disappeared prior to 3 months of age in 4-9 infants, and between 3 and 6 months in the other 5 infants. Three infants subsequently seroconverted and became virus position at 9-15 months of age. Milk samples from all mothers were virus negative at parturition, but milk samples from four animals were virus positive at nine and 12 months post-partum. Two of the three infected infants have died (7 and 9 months from time infection was documented), and the other infant is showing lymphadenopathy and progressive immunosuppression. This is the first documentation of maternal-infant transmission of SIV and also represents the first isolation of SIV from the milk of infected macaques. Our observation suggest that maternal-infant transmission occurred by breast-feeding.

SPECIFIC AIM 3e.: Serological Survey of Non-Human Primates in Kenya

In ongoing studies, serological surveys are being conducted on serum samples provided from feral non-human primates (baboons, Sykes monkeys and African green monkeys) in Kenya. These samples are checked for antibodies to SIV, STLV-1 and HIV. The following is a summary of these serological findings to date:

	Seropositive/Number of Animals Checked		
	SIV/HIV-2	HIV-1	STLV-1/HTLV-1
African Greens	279/542 (51%)	114/542 (21%)	231/541 (43%)
Baboons	1/373 (0.2%)	11/373 (3%)	20/373 (5%)
Sykes Monkeys	71/120 (59%)	21/120 (18%)	36/120 (30%)

Due to the high seropositive rate for antibodies to SIV in Sykes monkeys, arrangements were made to have nine (6 seropositive, 3 seronegative) Sykes monkeys shipped to the Yerkes Center to attempt virus isolation. An SIV was isoldated from these Sykes monkeys; this is summarized in the next section of this report.

SPECIFIC AIM 3f.: Isolation of a Lentivirus from African Sykes Monkeys

As previously noted, a lentivirus, designated SIVsyk, was isolated from five of six seropositive, asymptomatic Sykes monkeys, although in four cases, isolation was successful only after depletion of CD8+ lymphocytes and cocultivation of the CD4+ cell population with peripheral blood mononuclear cells from seronegative Sykes monkeys. SIVsyk resembles other SIVs morphologically, has a MG^{2+}-dependent reverse transcriptase enzyme and replicates in and is cytopathic for CEMx174 and SupT1 cells, suggesting that the virus is tropic for CD4+ cells. SIVsyk differs from other SIVs in that it failed to replicate in normal human, mangabey and macaque PBMC. This isolate is currently being cloned and sequenced by Drs. V. Hirsch and P. Johnson. To date, sequence analysis has revealed that the SIVsyk gag region is distantly related to the other reported SIV sequences; 50% amino acid identity was observed between SIVsyk and either SIVsmm, or two divergent SIVagm isolates. This identity is lower than that observed between SIVsmm and SIVagm (605) due to a 25 amino acid insertion near the amino terminus of the gag protein of SIVsyk. In addition, by nucleotide comparison, SIVsyk is only distantly related to SIV from mandrills (SIVmnd) and HIV-1. SIVsyk, therefore, is a novel member of the SIV family of viruses, distinct from all other strains previously described. Rhesus and pig-tailed macaques will be inoculated with SIVsyk in the near future to determine its pathogenicity and pathogenesis in these species.

SPECIFIC AIM 3g. Mangabey, Rhesus and Pig-tailed Macaque Breeding Colonies

The mangabey, rhesus and pig-tailed macaque breeding colonies dedicated to the support of AIDS animal model related studies now number 209 rhesus, 194 pigtails, and 147 mangabeys (these numbers include all animals in the colony from infants to adult breeders); 137 of the pigtails are recent additions to the colony. The rhesus and mangabey breeding colonies continue to reproduce very successfully; the productivity of the pig-tailed macaque breeding colony has been less successful. The productivity of the pigtail colony should be increased substantially due to the recent addition of 137 newly imported animals. The productivity of these breeding colonies is summarized below:

Summary of AIDS Animal Model Breeding Colonies
January 1, 1990 through December 31, 1990

Mangabey	
Total Births	31
Stillborn/Neonatal Loss	5
Surviving	26
Pigtail	
Total Births	29
Stillborn/Neonatal Loss	11
Surviving	18
Rhesus	
Total Births	67
Stillborn/Neonatal Loss	10
Surviving	57

Publications (1986-1990) Related to AIDS Animal Model Program

Fultz, P.N., McClure, H.M., Anand, R., and Srinivasan, A.: Isolation of a T-lymphotropic retrovirus from health mangabey monkeys. J. Cell Biochem. Suppl. 19A:188 (1986) (Abstract).

Fultz, P.N., McClure, H.M., Anderson, D.C., Swenson, R.B., Anand, R., and Srinivasan, A.: Isolation of a T-lymphotropic retorvirus from naturally infected sooty mangabey monkeys (Cercocebus atys). Proc. Natl. Acad. Sci. 83:5286-5290 (1986).

Fultz, P.N., McClure, H.M., Anand, R., and Srinivasan, A.: Isolation of a T-lymphotropic retorvirus from healthy mangabey monkeys. In: Viruses and Human Cancer, UCLA Symp. Molec. Cell. Biol., Vol. 43, edited by R.D. Gallo, W. Haseltine, F. Klein and H. Zur Hausen, Alan R. Liss, Inc., New York, pp. 297-308, 1987.

Fultz, P.N., McClure, H.M., and Anderson, D.C.: STLV-III/SMM-specific antibodies in persistently-infected mangabey monkeys and acutely-infected rhesus macaques. In: Proceedings International Conference on AIDS, Paris, France, p. 10, June 23-25, 1986.

McClure, H.M.: Mangabey T-lymphotropic retrovirus (SIV/SMM): Isolation, serology and experimental infection. Presented at the 1986 Symposium on Simian AIDS and related disorders, Yountville, California, September 21-24, 1986.

Fultz, P., McClure, H., Switzer, W., McGrath, N., Swenson, F., and Srinivasan, A.: Development of animal models to test potential vaccines for protection against infection by T-lymphotropic retroviruses. In: Modern Approaches to New Vaccines, Including Prevention of AIDS, Cold

Spring Harbor Laboratory, Cold Springs Harbor, N.Y., p. 38, 1986 (Abstract).

Fultz, P.N., Switzer, W., McGrath, N., Srinivasan, A., McClure, H.M., Brodie, A., and Swenson, B.: Animal models to test potential vaccines for protection against infection by T-lymphotropic retroviruses. In: Vaccines 87, edited by Chanock, Brown, Lerner, Ginsberg, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 205-208, 1987.

Anderson, D., Fultz, P., and McClure, H.: Necrotizing gingivitis (NOMA) in an SIV/SMM infected neonatal mangabey monkey. In: Proceedings of International Conference on AIDS in Children, adolescents and heterosexual adults: an interdisciplinary approach to prevention. Atlanta, Georgia, February 19-21, 1987 (Abstract).

McClure, H., Fultz, P., and Anderson, D.: SIV/SMM infected macaques as models for HIV infection. In: Proceedings of International Conference on AIDS in Children, Adolescents, and Heterosexual Adults: An Interdisciplinary Approach to Prevention. Altanta, Georgia, February 19-21, 1987 (Abstract).

Fultz, P.N., Switzer, W., Greene, N., Mullally, R., and McClure, H.: Comparative analysis of antibody responses of nonhuman primates to infection by human and simian T-lymphotropic retroviruses. J. Cell. Biochem. Suppl. 11D:42 (1987) (Abstract).

McClure, H.M., Anderson, D.C., Switzer, W.M., Strobert, E.A., Orkin, J.L., and Fultz, P.N.: SIV/SMM: Prevalence and association with disease in a breeding colony of mangabey monkeys. In: Proceedings III International Conference on AIDS, Washington, D.C., p.14, June 1-5, 1987 (Abstract).

McClure, H.M., Anderson, D.C., Swenson, R.B., Orkin, J., Strobert, E.A., and Fultz, P.N.: Response of pig-tailed macaques to SIV/SMM infection. In: Proceedings III International Conference on AIDS, Washington, D.C., p.212, June 1-5, 1987 (Abstract).

Fultz, P., Switzer, W., McClure, H., Reed, C., and Montagnier, L.: *Simian models for AIDS: SIV/SMM and HIV-2 infection of rhesus macaques.* In: Modern Apporaches to Vaccines and Prevention of AIDS, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., September 9-13, 1987 (Abstract).

Anderson, D., McClure, J., and Fultz, P.: Pathology of SIV/SMM infections in mangabeys and chronically infected rhesus macaques. Abstracts: Symposium on nonhuman primate models for the Acquired Immunodeficiency Symdrome (AIDS), Atlanta, Georgia, October 8-10, 1987, p. 8.

McClure, H., Anderson, D., and Fultz, P.: Response of Pig-tailed Macaques to SIV/SMM infection. Abstracts: Symposium on Nonhuman

Primate Models for the Acquired Immunodeficiency Syndrome (AIDS), Atlanta, Georgia, October 8-10, 1987, p. 11.

Fultz, P., Anderson, D., and McClure, H.: Characterization of a Variant of SIV/SMM. Abstracts: Symposium on Nonhuman Primate Models for the Acquired Immunodeficiency Syndrome (AIDS), Atlanta, Georgia, October 8-10, 1987, p. 14.

Ansari, A.A., Brodie, A., Fultz, P., and McClure, H.: Identification of a Panel of Monoclonal Reagents and Dual Color Flow Microfluorometry for the Study of Primate Mononuclear Cells and In Vitro SIV Specific T Cell Proliferation Assay. Anstracts: Symposium on Nonhuman Primate Models for the Acquired Immunodeficiency Syndrome (AIDS), Atlanta, Georgia, October 8-10, 1987, p. 16.

Fultz, P., Switzer, W., McClure, H., Anderson, D., and Montagnier, L.: Infection of Rhesus Macaques with HIV-2. Abstracts: Symposium on Nonhuman Primate Models for the Acquired Immunodeficiency Syndrome (AIDS), Atlanta, Georgia, October 8-10, 1987, p. 32.

Fultz, P.N., Switzer, W.M., Anderson, D.C., and McClure, H.M.: Generation of a Virulent Strain of SIV/SMM During Passage in a Pig-tailed Macaque. VIIth International Congress of Virology, Edmonton, Alberta, Canada, August 8-14, 1987, p. 165 (Abstract).

Fultz, P.N., Switzer, W., McClure, H.M., Anderson, D., and Montagnier, L.: Simian Models for *AIDS: SIV/SMM and HIV-2 Infection of Macaques.* Vaccines 88, Cold Spring Harbor Laboratory, pp. 167-170, 1988.

Fultz, P.N., Switzer, W., Horaist, C., McClure, H., and Wain-Hobson, S.: Characterization of a Variant of SIV/SMM with Increased Virulence. IV International Conference on AIDS, Stockholm, Sweden, June 12-16, 1988.

Ahmed-Ansari, A., Powell, J.D., Fultz, P.N., Anderson, D., Sell, K.W., and McClure, H.: Viral Antigen Processing Requirement for the In Vitro T Cell Specific Proliferative Response to SIV/SMM. In: Symposium on Nonhuman Primate Models for AIDS, November 16-18, 1988, San Antonio, Texas, p. 40 (Abstract).

Powell, J.D., Ahmed-Ansari, A., Fultz, P.N. Anderson, D., Sell, K.W., and McClure, H.M.: Phenotypic and Functional Differences in NK and LAK Cells from Naturally SIV/SMM Infected and Non-Infected Sooty Mangabeys and Normal Rhesus Macaques. In: Symposium on Nonhuman Primate Models for AIDS, November 16-18, 1988, San Antonio, Texas, p. 41 (Abstract).

Anderson, D., McClure, H., Ansari, A., and Fultz, P.: Spectrum of Disease in Macaques Chronically Infected with SIV/SMM. In: Symposium on Nonhuman Primate Models for AIDS, November 16-18, 1988, San Antonio, Texas, p. 46 (Abstract).

McClure, H., Anderson, D., Ansari, A., and Fultz, P.: Update on the Pathogenicity of SIV/SMM/PBj14, An Acutely Lethal Variant of SIV/SMM.

In: Symposium on Nonhuman Primate Models for AIDS, November 16-18, 1988, San Antonio, Texas, p. 47 (Abstract).

McClure, H.M., Anderson, D.C., Ansari, A.A., and Fultz, P.N.: Clinicopathologic Findings and Nonhuman Primates Infected with an Acutely Lethal Variant of SIV/SMM. Lab. Invest. 60: 60A (1989) (Abstract).

McClure, H.M., Anderson, D.C., Ansari, A.A., and Fultz, P.N.: Pathology of Chronic SIV/SMM Infection in Macaque Monkeys. Lab. Invest. 60: 60A (1989) (Abstract).

McClure, H.M., Anderson, D.C., Fultz, P.N., Ansari, A.A., Lockwood, E., and Brodie, A.: Spectrum of Disease in Macaque Monkeys Chronically Infected with SIV/SMM. Vet. Immunol. Immunopathol. 21:13-24 (1989).

Ansari, A.A., Brodie, A.R., Fultz, P.N., Anderson, D.C., Sell, K.W., and McClure, H.M.: Flow microfluorometric analysis of peripheral blood mononuclear cells from nonhuman primates: correlation of phenotype with immune function. Am. J. Primatol. 17:107-131 (1989).

Fultz, P., Stricker, R., Montagnier, L., Sonigo, P., McClure, H., and Anderson, D.: Role of antibodies, cytokines, and virus variation in SIV/SMM induced disease. In: Proceedings V International Conference on AIDS, Montreal, Canada, p.511, June 4-9, 1989.

McClure, H.M., Anderson, D.C., Fultz, P., Ansari, A., Brodie, A., and Lehrman, S.: Prophylactic effects of AZT following exposure of macaques to an acutely lethal variant of SIV (SIV/SMM/PBj-14). In: Proceedings V International Conference on AIDS, Montreal, Canada, p.522, June 4-9, 1989 (Abstract).

Anderson, D.C., McClure, H.M., Ribas, J.L., Ansari, A., Fultz, P., and Mirra, S.: Neuropathological Study of SIV-Infected macaques. In: Proceedings V International Conference on AIDS, Montreal, Canada, p.532, June 4-9, 1989 (Abstract).

McClure, H.M., Anderson, D.C., Ansari, A., and Fultz, P.: In vivo analysis of the minimal infectious dose of an acutely lethal pool of SIV (SIV/SMM/PBj-14). In: Proceedings V International Conference on AIDS, Montreal, Canada, p. 594, June 4-9, 1989 (Abstract).

Ribas, J.L., Baskin, G., King, N.W., McClure, H.M., Lowenstine, L.J., and Axthelm, M.L.: Development of a study set on the pathology of simian retroviral diseases. In: Proceedings V International Conference on AIDS, Montreal, Canada, p. 616, June 4-9, 1989 (Abstract).

Fultz, P., McClure, H., and Anderson, D.: Replication of a lethal variant of SIV/SMM in macaque PBMC in the absence of exogenous IL-2 or activation by mitogens. In: Proceedings V International Conference on AIDS, Montreal, Canada, p. 684, June 4-9, 1989 (Abstract).

Powell, J.D., Jehuda-Cohen, T., McClure, H.M., Anderson, D., Fultz, P.N., Sell, K.W., and Ansari, A.A.: Phenotypic and functional differences in NK and LAK cells from SIV infected asymptomatic mangabeys and normal macaques: potential protective role in SIV infection, FASEB Journal 3:A1327 (1989)(Abstract).

Powell, J.D., McClure, H.M., Anderson, D., Fultz, P.N., Sell, K.W., Ahmed-Ansari, A.: Phenotypic and functional differences in NK and LAK cells in the peripheral blood of sooty mangabeys and rhesus macaques. Cell Immunol. 124:107-118 (1989).

Jehuda-Cohen, T., Powell, J.D., McClure, H.M., Anderson, D., Fultz, P.N., Sell, K.W., and Ansari, A.A.: Antigen processing requirements for the in vitro proliferation to SIV by T-call from naturally infected mangabeys and SIV infected macaques. FASEB Journal 3:A961 (1989) (Abstract).

McClure, H., Anderson, D., Fultz, P., and Ansari, A.: Nonhuman primate models for AIDS research. International Symposium on Biomedical Research Strategy on AIDS, Yverdon-les-Bains, Switzerland, September 24-28, 1989 (Abstract).

Villinger, F., Jehuda-Cohen, T., Powell, J.D., Anderson, D., McClure, H.M., and Ansari, A.A.: Relative differences in the regulation and susceptibility for the replication of SIV/SMM in CD4+ T cells and monocytes from asymptomatic mangabeys and disease susceptible rhesus *macaques. 7th Annual Symposium on Nonhuman Primate Models for* AIDS, Portland, Oregon, October 11-13, 1989, p. 42 (Abstract).

McClure, H.M., Anderson, D.C., Brodie, A., Lockwood, E., Fultz, P., Ansari, A., and Gordon, T.: T-cell leukemia in a mangabey monkey from a colony with a high incidence of SIV and STLV-1 infection. 7th Annual Symposium on Nonhuman Primate Models for AIDS, Portland, Oregon, October 11-13, 1989, p. 61 (Abstract).

Ansari, A.A., Powell, J.D., Jehuda-Cohen, T., Villinger, F., Anderson, D., and McClure, H.M.: Delineation of peptide specific T cell immunity in SIV/SMM infected nonhuman primates using the cellular western blot assay. 7th Annual Symposium on Nonhuman Primate Models for AIDS, Portland, Oregon, October 11-13, 1989, p. 70 (Abstract).

Powell, J.D., Jehuda-Cohen, T., Villinger, F., Anderson, D., McClure, H.M., and Ansari, A.A.: CD8+ T cells from SIV seronegative sooty mangabeys suppress SIV/SMM virus replication in vitro. 7th Annual Symposium on Nonhuman Primate Models for AIDS, Portland, Oregon, October 11-13, 1989, p. 72 (Abstract).

Jehuda-Cohen, T., Slade, B., Powell, J.D., Villinger, F., Anderson, D., McClure, H.M., Sell, K.W., and Ansari, A.A.: A novel method for the detection of antibodies against SIV and HIV in conventionally seronegative sooty mangabeys and high risk humans. 7th Annual Symposium

on Nonhuman Primate Models for AIDS, Portland, Oregon, October 11-13, 1989, p. 76 (Abstract).

McClure, H.M., Anderson, D., Fultz, P., and Ansari, A.: SIV/SMM-infected macaque monkeys as models for AIDS. International TNO Meeting on Animal Models in AIDS, Maastricht, The Netherlands, October 23-26, 1989, p. 60-61 (Abstract).

Fultz, P.N., Stricker, R.B., McClure, H.M., Anderson, D.C., Switzer, W., and Horaist, C.: The role of antibodies in protection from or potentiation of disease in SIV/SMM-infected mangabeys and macaques. International TNO Meeting on Animal Models in AIDS, Maastricht, The Netherlands, October 23-26, 1989, p. 20-21 (Abstract).

Ribas, J.L., Schlessinger, R., Anderson, D.C., McClure, H.M., and Mirra, S.: Immunocytochemical and histopathological study of meningoencephalitis in macaque monkeys induced by simian immunodeficiency virus. International TNO Meeting on Animal Models in AIDS, Maastricht, The Netherlands, October 23-26, 1989, p. 46 (Abstract).

Fultz, P.N., McClure, H.M., Anderson, D.C., Siegel, R.L., Ansari, A., and Brodie, A.: Changes in antibody profiles and hematologic parameters associated with long-term HIV-1 infection in chimpanzees. International TNO Meeting on Animal Models in AIDS, Maastricht, The Netherlands, October 23-26, 1989, p. 55-56 (Abstract).

Jahrling, P.B., Lewis, M.G., Zack, P.M., Fultz, P.N., McClure, H.M., and Peters, C.J.: Infection of rhesus macaques with a rapidly fatal sooty mangabey SIV isolate (SIV/SMM/Pbj). International TNO Meeting on Animal Models in AIDS, Maastricht, The Netherlands, October 23-26, 1989, p. 56-57 (Abstract).

McClure, H.M., Anderson, D.C., Fultz, P., Ansari, A., and Schinazi, R.: Nonhuman primate models for AIDS therapy. Second International Conference on Drug Research in Immunologic and Infectious Diseases: Acquired Immune Deficiency Syndrome (AIDS), Arlington, Virginia, November 6-9, 1989, Abstract #24 (Abstract).

Fultz, P.N., Anderson, D.C., McClure, H.M., Sonigo, P., Montagnier, L., and Stricker, R.B.: Virulence and Variation of Viruses: Coalition with Cells, Cytokines and Simians. AIDS Res. Human Retroviruses 6:70 (1990) (Abstract).

Fultz, P.N., Stricker, R.B., McClure, H.M., Anderson, D.C., Switzer, W.M., and Horaist, C.: Humoral Response to SIV/SMM Infection in Macaque and Mangabey Monkeys. J. Acquired Immune Deficiency Syndromes 3:319-329 (1990).

Fultz, P.N., Gordon, T.P., Anderson, D.C., and McClure, H.M.: Prevalence of Natural Infection with Simian Immunodeficiency Virus and

Simian T-Cell Leukemia Virus Type I in a Breeding Colony of Sooty Mangabey Monkeys. AIDS 4:619-626 (1990).

Ahmed-Ansari, A., Powell, J.D., Jensen, P.E., Jehuda-Cohen, T., McClure, H.M., Anderson, D., Fultz, P.N., and Sell, K.W.: Requirements for Simian Immunodeficiency Virus Antigen-Specific In Vitro Proliferation of T Cells from Infected Rhesus Macaques and Sooty Mangabeys. AIDS 4:399-408 (1990).

Novembre, F.J., Hirsch, V.M., McClure, H.M., and Johnson, P.R.: Molecular Diversity of SIVsm/Pbj and a Cognate Viriant (SIVsm/PGg). 8th Annual Symposium on Nonhuman Primate Models for AIDS, November 28-30, 1990, New Orleans, Louisiana, p. 17 (Abstract).

Villinger, F., De, B., Jehuda-Cohen, T., Powell, J.D., Neckelmann, N., McClure, H.M., Folks, T.M., and Ansari, A.A.: Both in Vitro Culture and In Vivo Passage Lead to Selective Replication of SIV Variants. 8th Annual Symposium on Nonhuman Primate Models for AIDS, November 28-30, 1990, New Orleans, Louisiana, p. 18 (Abstract).

McClure, H.M., Anderson, D.C., Fultz, P.N., Ansari, A.A., Jehuda-Cohen, T., Villinger, F., Klumpp, S.A., Switzer, W., Lockwood, E., and Brodie, A.: Maternal Transmission of SIVsmm in Rhesus Macaques. 8th Annual Symposium on Nonhuman Primate Models for AIDS, November 28-30, 1990, New Orleans, Louisiana, p. 34 (Abstract).

Jehuda-Cohen, T., Villinger, F., McClure, H.M., Lockwood, E., Powell, J.D., and Ansari, A.A.: Transmission of SIV Infection by Transfusion of Seronegative Blood in Nonhuman Primates. 8th Annual Symposium on Nonhuman Primate Models for AIDS, November 28-30, 1990, New Orleans, Louisiana, p. 37 (Abstract).

Hillyer, C.D., Brodie, A.R., Ansari, A.A., Anderson, D.C., and McClure, H.M.: Autoimmune Hemolytic Anemia in SIVsmm Infected Macaca mulatta. 8th Annual Symposium on Nonhuman Primate Models for AIDS, November 28-30, 1990, New Orleans, Louisiana, p. 39 (Abstract).

Powell, J.D., Bednarik, D.P., Folks, T.M., Jehuda-Cohen, T., Villinger, F., McClure, H.M., and Ansari, A.A.: CD8+ T-Cell-Induced Inhibition of Retroviral Replication is Mediated at the Transcriptional Level. 8th Annual Symposium on Nonhuman Primate Models for AIDS, November 28-30, 1990, New Orleans, Louisiana, p. 43 (Abstract).

Fomsgaard, A., Hirsch, V.M., McClure, H.M., Novembre, F.J., and Johnson, P.R.: Cloning and Expression of Primate CD4 Molecules: Diversity of the Cellular Receptor for SIV/HIV. 8th Annual Symposium on Nonhuman Primate Models for AIDS, November 28-30, 1990, New Orleans, Louisiana, p. 48 (Abstract).

Ribas, J.L., Sesterhenn, I.A., McClure, H.M., Anderson, D.C., Frizzera, G., and Benton, C.S.: Preliminary Observations on the Immunophenotyping of Mononuclear Cells and the Detection of Viral Antigens Using Formalin-

Fixed, Parafin-Embedded Sections from SIV-Infected Macaque Monkeys. 8th Annual Symposium on Nonhuman Primate Models for AIDS, November 28-30, 1990, New Orleans, Louisiana, p. 85 (Abstract).

Vuchetich, M., Jehuda-Cohen, T., Villinger, F., Powell, J.D., McClure, H.M., and Ansari, A.A.: Evidence that the Seronegative Sooty Mangabeys in Our Colony are Infected with SIVsmm. 8th Annual Symposium on Nonhuman Primate Models for AIDS, November 28-30, 1990, New Orleans, Louisiana, p. 98 (Abstract).

Ansari, A.A., Jehuda-Cohen, T., Powell, J.D., Villinger, F., Mayne, A., Lockwood, E., Gordon, T., and McClure, H.M.: Cellular and Cytokine Requirements for the In Vitro Synthesis of SIV-Reactive Antibodies by Polyclonal Activation of PBMC from Infected yet Seronegative Nonhuman Primates. 8th Annual Symposium on Nonhuman Primate Models for AIDS, November 28-30, 1990, New Orleans, Louisiana, p. 108 (Abstract).

Klumpp, S.A., Anderson, D.C., Fultz, P.N., Ribas, J.L., Ansari, A.A., and McClure, H.M.: Disease in Mangabey Monkeys Associated with Simian Immunodeficiency Virus and Simian T-lymphotropic Virus Type 1. 8th Annual Symposium on Nonhuman Primate Models for AIDS, November 28-30, 1990, New Orleans, Louisiana, p. 129 (Abstract).

Emau, P., McClure, H.M., Isahakia, M., Else, J., and Fultz, P.: Isolation from African sykes Monkeys (Cercopithecus mitis) of a Lentivirus Related to Human and Simian Immunodeficiency Viruses. 8th Annual Symposium on Nonhuman Primate Models for AIDS, November 28-30, 1990, New Orleans, Louisiana, p. 132 (Abstract).

Anderson, D.C., Fultz, P.N., Ansari, A.A., Klumpp, S.A., Ribas, J.L., and McClure, H.M.: Naturally Occurring SIV Infection in a Colony of Macaca arctoides. 8th Annual Symposium on Nonhuman Primate Models for AIDS, November 28-30, 1990, New Orleans, Louisiana, p. 133 (Abstract).